

1/20/98

1/20 STNFR ELISA - Mouse Serum of C3H mice injected with
 * L929 clone 39 + Primary tumor cell lines
 of C39.

+ Standard STNFR protocol
 + samples diluted in 0.1% BSA PBS/Queer
 + pNPP ~20min

	1	2	3	4	5	6	7	8	9	10	11	12
A	CLK unrad #1 1:5	CLK unrad #2 1:5	CLK unrad #3 1:5	CL39 unrad #1 1:5	CL39 unrad #2 1:5	CL39 unrad #3 1:5	CL39 3.0 Gy #1 1:5	CL39 3.0 Gy #2 1:5	CL39 3.0 Gy #3 1:5	CL39 3.0 Gy #4 1:5	TNFBPI 40ng/ml	
B												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.176	0.180	0.187	0.144	0.136	0.135	0.149	0.184	0.170	0.178	0.186	0.187
B	0.179	0.186	0.180	0.157	0.146	0.107	0.106	0.140	0.140	0.139	0.188	0.186
C	0.186	0.187	0.187	0.164	0.160	0.147	0.136	0.124	0.118	0.094	0.117	0.147
D	0.184	0.186	0.183	0.159	0.179	0.110	0.177	0.168	0.177	0.135	0.150	0.177
E	0.187	0.185	0.182	0.144	0.164	0.131	0.101	0.156	0.140	0.099	0.158	0.103
F	0.107	0.170	0.106	0.195	0.120	0.146	0.114	0.183	0.199	0.076	0.071	0.143
G	0.188	0.187	0.180	0.159	0.166	0.078	0.146	0.140	0.121	0.139	0.137	0.100
H	0.184	0.176	0.178	0.118	0.107	0.118	0.179	0.145	0.199	0.130	0.137	
F	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	96 sup	96 sup
G	CLK 5.0 #1 1:5	CLK 5.0 #2 1:5	CLK 5.0 #3 1:5	CL39 5.0 #1 1:5	CL39 5.0 #2 1:5	CL39 5.0 #3 1:5	CL39 5.0 #4 1:5	CL39 5.0 #1 1:5	CL39 5.0 #2 1:5	CL39 5.0 #3 1:5	CL39 5.0 #4 1:5	CL39 5.0 #1 1:5
H	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1/2	1/2	1/2	1/2	CL39 5.0 #1 1:5

			mean A405	mouse Serum	
CLK	1:5	unrad	1.0 Gy	3.0 Gy	5.0 Gy
	1:10	0.171	0.173	0.214	0.278
		0.175	0.240	0.245	0.173
CL39	1:5	0.272	0.123	0.162	0.172
	1:10	0.203	0.173	0.192	0.205

do Circulate
 STNFR being
 bound by
 circulating
 α-STNFR Ab

mean A405 L929 Clone 39 Primary tumor cell lines from C3H mice:

1.0 Gy - #5 = 0.801	5.0 Gy #1 = 0.815
3.0 Gy - #1 = 0.701	5.0 Gy #2 = 0.760
3.0 Gy - #2 = 0.620	5.0 Gy #3 = 0.650
3.0 Gy - #5 = 0.717	5.0 Gy #4 = 0.701

7/9/98

7/9 Determination of total α -STNFR Ab in the Circulation
 of mice immunized with Denatured STNFR (see
 pg 37 for info)

Groups of 5 mice each were immunized with denatured
 STNFR, PBS, or CFA alone and then challenged with
 1A29 Clone 39 tumor cells. Blood was collected at time
 of necropsy. Samples were heated to 37°C for 10min,
 spun in microfuge for 10min at 3000 rpm, and the
 serum was collected.

Assay

- 1) Coat with 2 μ g/ml of goat α -STNFR Ab - BLOCK
- 2) Add 0.5 μ g/ml rHSTNFR
- 3) Add diluted mouse serum
- 4) Add 2 μ g/ml goat α -mouse IgG, A, M - Phosphatase labelled
- 5) Add pNPP.

	1	2	3	4	5	6	7	8	9	10
A	#1 1/400	#2	#3	#4	#5 D	PBS #4 1/25	1/50	1/100	NMS 1/400	NMS alone (no STNFR)
B	1/800					PBS #5 1/25	1/50	1/100	1/800	
C	1/1600					PBS #1 1/25	1/50	1/100	1/1600	
D	1/3200	X				PBS #2 1/25	1/50	1/100	1/3200	
E	1/6400					PBS #3 1/25	1/50	1/100	1/6400	
F	1/12800					PBS #4 1/25	1/50	1/100	1/12800	
G	PBS #1 1/25	PBS 1/50	1/100	PBS #3 1/25	1/50	PBS #5 1/25	1/50	1/100	2 nd alone no STNFR	
H	PBS #2 1/25	1/50	1/100	1/100	#2 1/3200	NMS 1/25	1/50	1/100	STNFR alone (no STNFR)	

	1	2	3	4	5	6	7	8	9	10
A	0.046	0.070	0.106	0.144	0.181	0.078	0.046	0.038	0.007	0.110
B	0.068	0.094	0.119	0.149	0.190	0.087	0.047	0.038	0.010	0.110
C	0.077	0.088	0.097	0.105	0.108	0.170	0.070	0.034	0.007	0.110
D	0.088		0.088	0.094	0.111	0.145	0.088	0.057	0.007	0.110
E	0.094	0.098	0.098	0.070	0.047	0.075	0.055	0.074	0.007	0.110
F	0.070	0.075	0.075	0.070	0.070	0.071	0.044	0.074	0.007	0.110
G	0.106	0.087	0.081	0.071	0.053	0.071	0.044	0.077	0.007	0.110
H	0.145	0.075	0.077	0.045	0.047	0.050	0.050	0.037	0.000	0.110
I	0.090	0.074	0.097	0.074	0.070	0.070	0.070	0.070	0.070	0.110

9/4/98

9/4 - ID3 B1 Antitumor Infusion + Challenge w/ Clone 39

These experiments are designed to challenge the efficacy of ID3 B1 (an α -STNFR1 Ab) in protecting mice from Clone 39-derived B29 tumors. Mice will be injected with ID3 B1 or control Ab on day 0 (the time of tumor challenge) and again on days 3 and 6. Tumor development will be monitored daily.

9/4 (day 0) Inject ID3 B1 or 1B7.11 (α -TNP Ab) IP - 0.5 mg Ab in 1 ml PBS mouse
 ~ 11:00 AM using a 26 gauge needle (aliquotted using a pipetman, then inject all)
 ~ 2:30 PM Inject Clone 39 Subcutaneously (mid-back) 10⁶ cells 10.5 μ l of 10⁶ cells in 10.5 μ l of PBS
 26 gauge needle (aliquotted using a pipetman, then inject all)

9/7 (day 3) Check mice for tumors - none at this time
 Inject mice with the appropriate antitumor IP
 0.5 ml containing 0.2 mg Ab in PBS

9/9 (day 5) Check for tumors:
 ID3 B1 injected mice = 4 tumors
 1B7.11 " " = 8 tumors

9/10 (day 6) Check for tumors:
 ID3 B1 = 8
 1B7.11 = 8

Inject mice with the appropriate Ab IP
 0.5 ml containing 0.2 mg Ab in PBS

* note: the 1B7.11 mice actually got a total of 85 μ g of Ab - vs - 90 μ g of ID3 B1 Ab mice.

9/10 (day 7) ID3 B1 = 8
 1B7.11 = 8

9/12 (day 8) Same as above
 Resulted 2 tumors from 1B7.11 group + 1 tumor ID3 B1 group in

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all tumors are regressing.

26. need to optimize dosage, route of administration,
frequency of administration, etc.

end of Ph.D. work - all Book #5 for Post-DOC
Research

3/24/98

3/24 2-5TNFRI ELISA of Purified ID3 and Clones of Limiting Dilution

- * Test fractions of ID3 purified from protein G column
- * Test 1st set of clones from limiting dilution

Procedure

- Coat w/ 2 µg/ml goat α hTNFRI
- Block
- Add AB Supt (14937 + anti-mouse hTNFRI)
- Add appropriate dilut of test sample
- Add 2 µg/ml goat α mouse IgG, IgA, IgM - AP conjugate
- Develop as per usual
- PNPP incubated for ~5 hours at RT (the substrate was the old stuff + some of new stuff - Reaction was very poor)

	1	2	3	4	5	6	7	8	9	10	11
A	SPT Nat	Wash #1 Nat	Wash #2 Nat	Wash #3 Nat	Wash #4 Nat	Wash #5 Nat	SAL #2 2 µg/ml	SAL #3 2 µg	SAL #4 2 µg	SAL #5 2 µg	SAL #6 2 µg
B	1/2	1/2	1/2	1/2	1/2	1/2	4 µg	4 µg	4 µg	4 µg/ml	4 µg
C	1/4	1/4	1/4	1/4	1/4	1/4	SPT Supt + binding buffer	SAL 4 µg Supt + buffer	SPT Supt alone (blank)		
D	4F2 7F	4F2 4F	4F2 10C	4F2 10C	4F2 B1	4F2 A12	147 F2 (blank)	3B11 A2	3B11 B12	3B11 B8	3B11 F7
E	2B8 B12	1A8 B9	1A8 C2	1A8 D4	1A8 D5	1A8 E9	1A8 F10	1A8 F5	1A8 G8	147 D2	4F2 D0

2 spots
in 50%
Supt above

	1	2	3	4	5	6	7	8	9	10	11
A	0.363	0.447	0.081	0.060	0.038	0.044	1.404	1.600	1.571	1.657	1.657
B	0.897	1.260	0.078	0.039	0.032	0.039	1.456	1.316	1.412	1.472	1.572
C	0.917	1.157	0.106	0.066	0.053	0.030			0.065	0.000	
D	0.426	0.420	0.066	0.133	0.065	0.060	0.041	0.041	0.041	0.037	0.032
E	0.080	0.075	0.778	0.304	0.402	0.455	1.520	0.246	0.256	0.146	

Patched 4F2-7F, 4F, 10C and 1A8 - C2, D4, D5, E9, F10, F5, G8
and 147 - D2

notes: purified Ab is biologically active (in terms of binding to native TNF). Concentration may be much lower than predicted.

3B11 may be a total bust - no positive clones and poor binding to 9B in previous assay (pg. 29)